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Molecular phylogeny of the forensically important genus *Cochliomyia* (Diptera: Calliphoridae)

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Abstract

Cochliomyia Townsend includes several abundant and one of the most broadly distributed, blow flies in the Americas, and is of significant economic and forensic importance. For decades, *Cochliomyia hominivorax* (Coquerel) and *C. macellaria* (Fabricius) have received attention as livestock parasites and primary indicator species in forensic entomology. However, *C. minima* Shannon and *C. aldrichi* Del Ponte have only been subject to basic taxonomy and faunistic studies. Here we present the first complete phylogeny of *Cochliomyia* including numerous specimens per species, collected from 13 localities in the Caribbean. Four genes, the mitochondrial COI and the nuclear EF-1α, 28S rRNA, and ITS2, were analyzed. While we found some differences among gene trees, a concatenated gene matrix recovered a robustly supported monophyletic *Cochliomyia* with *Compsomyiops* Townsend as its sister group and recovered the monophyly of *C. hominivorax*, *C. macellaria* and *C. minima*. Our results support a close relationship between *C. minima* and *C. aldrichi*. However, we found *C. aldrichi* containing *C. minima*, indicating recent speciation, or issues with the taxonomy of the group. We provide basic information on habitat preference, distribution and feeding habits of *C. minima* and *C. aldrichi* that will be useful for future forensic studies in the Caribbean.

Keywords

Forensic entomology, Caribbean region, habitat preferences, *Cochliomyia minima*, *Cochliomyia aldrichi*, *Cochliomyia macellaria*, *Cochliomyia hominivorax*

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Introduction

Cochliomyia Townsend is endemic to the Americas and includes only four species: *Cochliomyia minima* Shannon, *C. aldrichi* Del Ponte, *C. macellaria* (Fabricius) and *C. hominivorax* (Coquerel). All of them are flesh eaters during their larval stage and are locally abundant. In particular, *Cochliomyia macellaria* is one of the most broadly distributed blow flies in the New World (Whitworth 2010). These species vary in habitat preference, feeding habits, dispersal abilities, and morphology among the species (Hall 1948, Whitworth 2010). For instance, *C. aldrichi*, *C. minima* and *C. macellaria* are primarily carrion feeders, while, *C. hominivorax* is an obligate parasite of mammals (Hall 1948, Stevens and Wallman 2006, McDonagh et al. 2009, McDonagh and Stevens 2011).

Cochliomyia hominivorax and *C. macellaria* have been intensely studied due to their commercial and forensic importance. *Cochliomyia macellaria* is one of the most forensically important species commonly found on decomposing remains. This species is considered important for post mortem interval estimations (Smith 1986, Byrd and Castner 2010) being among the first species to colonize corpses. In contrast, *Cochliomyia hominivorax* is an obligate parasite with its larvae producing myiasis and feeding on living tissue (Hall 1948, Guimaraes et al. 1983). This species is one of the most important insect pests of livestock in the Neotropics causing economic losses of billions of dollars every year (Vargas-Terán et al. 2005). Both species are common throughout the year in tropical, warm and humid areas (Hall 1948). *Cochliomyia macellaria* can be found in temperate climates from Canada to Argentina during the summer months (Whitworth 2010). *Cochliomyia hominivorax* initially ranged from southern United States to northern Argentina (Guimaraes et al. 1983) but has been eradicated from North America, Central America, Puerto Rico and the Virgin Islands (Vargas-Terán et al. 2005). It is worth noting that in 1988 this species was introduced in Libya and it was successfully eradicated in 1992 based on the sterile insect technique (SIT). This was the major international effort and avoid a major disaster for the livestock industry of Africa and Southern Europe (Lindquist et al. 1992). Despite those successfully eradications *C. hominivorax* continues to be an economically important pest in South America and parts of the Caribbean (Vargas-Terán et al. 2005).

The other two congeners, *C. minima* and *C. aldrichi*, are poorly known and research has been limited to descriptive morphology and faunistics (Hall 1948, Dear 1985, Whitworth 2010). These two species are restricted to the West Indies and *C. aldrichi* has been reported in the Florida Keys (Whitworth 2010). Dear (1985) listed *C. minima* for the Florida Keys, however Whitworth (2010) concluded that Dear mistakenly identified one *C. aldrichi* specimen as *C. minima*. Forensically important insects in the Caribbean are generally understudied and these two species have not played an important role in forensic entomology. Yet, due to their abundance and broad distribution in this region, including Cuba, Dominican Republic, Jamaica, Puerto Rico, Virgin Islands, Bahamas and Cayman Islands (Hall 1948, Dear 1985, Whitworth 2010) they have an enormous forensic potential. For example, recent studies conducted in

Puerto Rico showed that *C. minima* is abundant and widely distributed on the island, and that adults are attracted to, and feed on, carrion (Yusseff-Vanegas 2014).

Although the adult morphology of the four species is well known (Hall 1948, Dear 1985, Whitworth 2010), studies on the relationship among *Cochliomyia* species have not been conducted yet. Morphological studies have provided synapomorphies of *Cochliomyia* that clearly diagnose it from all other Calliphoridae (Hall 1948; Dear 1985; Whitworth 2010). These include short and filiform palpus and phallus with extremely elongated paraphallus and a complex distiphallus (Dear 1985, Figs 37–44). Prior studies on the relationships among Calliphoridae (Stevens 2003, Harvey et al. 2008, McDonagh and Stevens 2011) and the subfamily Chrysomyinae (Singh and Wells 2011), including *C. macellaria* and *C. hominivorax*, supported *Cochliomyia* monophyly, and placed it as sister to *Compsomyiops* Townsend. However, the monophyly of the genus has not been formally tested with thorough sampling of all species, and the relationships among its species remain unknown. Furthermore, DNA-based methods can provide reliable identification of specimens by non-experts and will be particularly important for the identification of larval stages of *C. minina* and *C. aldrichi* that remain poorly known. For example, only the third instar of *C. minima* has been described (Yusseff-Vanegas 2014).

Here we provide a robust phylogenetic hypothesis of *Cochliomyia* based on four genes sequenced from 38 individuals collected throughout the Caribbean, including for the first time molecular data about *C. minima* and *C. aldrichi*. Our main goals are to test the monophyly of this genus and the validity of, and relationships among, its species.

Methods

Specimens and DNA extraction

A total of 44 specimens were included in this study, 38 representing the ingroup plus six outgroup species [*Chrysomya megacephala* (Fabricius), *C. rufifacies* (Macquart), *Hemilucilia sp.*, *Lucilia cuprina* (Wiedemann), *Compsomyiops fulvicrura* (Robineau-Desvoidy) and *Compsomyiops callipes* (Bigot)]. All sequences used here are new except for *Compsomyiops fulviclura* and *C. callipes* (Table 1). The specimens were collected in the Caribbean (Jamaica, Cuba, Dominican Republic, Puerto Rico, Saint Barts Martinique and Dominica) from 2011 to 2013 and in the following countries, Colombia (2014), Florida (2013) and Mexico (2010 and 2012) (Table 1). All specimens were killed and preserved in 95% ethanol and stored at -20 °C. The adults were examined with a Leica MZ16 stereomicroscope and identified using the Whitworth (2010) keys. The DNA was isolated from thoracic muscle or two legs of each individual with the QIAGEN DNeasy Tissue Kit (Qiagen, Inc., Valencia, CA). Voucher specimens were deposited at the UVM Natural History Museum (in the Zadock Thompson Zoological Collections) and sequences were submitted to GenBank.

Table 1. Specimen details, collection information and GenBank accession numbers.

Species name –Voucher Number	Location	*CO1*	*EF-1α*	*ITS2*	*28S rRNA*
Cochliomyia macellaria CO002	Colombia, El refugio Dry Forest	KX529522	KX529616	KX529574	KX529487
Cochliomyia macellaria CO010	Colombia, Choco, Jardín botánico del Pacífico	KX529545	KX529617	KX529575	KX529488
Cochliomyia macellaria CO017	Colombia, Santander, Chipatá, Finca el Castillo	KX529543	KX529618	KX529576	KX529489
*Cochliomyia macellaria ME015**	Mexico, Torreon, Coahuila	KX529546	KX529629	KX529588	KX529492
Cochliomyia macellaria FL006	USA, Florida, Everglades National Park, North-east	KX529535	KX529623	KX529581	KX529503
Cochliomyia macellaria JA002	Jamaica, Marshall's Pen House	KX529538	KX529624	KX529582	KX529502
Cochliomyia macellaria CU018	Cuba, Pinar del Rio, Viñales Nacional Park	KX529526	KX529620	KX529578	KX529499
Cochliomyia macellaria CU014	Cuba, Pinar del Rio, Viñales Nacional Park	KX529541	KX529619	KX529577	KX529497
Cochliomyia macellaria DR134	Dominican Republic, Puerto Plata	KX529527	KX529622	KX529580	KX529504
Cochliomyia macellaria DR010	Dominican Republic, El Morro, Monte Cristi	KX529536	KX529621	KX529579	KX529496
Cochliomyia macellaria PR129	Puerto Rico, Vieques, Monte Pirata	KX529542	-	KX529591	KX529501
Cochliomyia macellaria PR128	Puerto Rico, Vieques, Monte Pirata	KX529540	-	KX529590	KX529494
Cochliomyia macellaria PR121	Puerto Rico, Trujillo Alto, Ciudad Universitaria	KX529544	KX529630	KX529589	KX529500
Cochliomyia macellaria M112	Puerto Rico, Isla de Mona, Los Caobos	KX529528	-	KX529587	KX529493
Cochliomyia macellaria M081	Puerto Rico, Isla de Mona, Los Caobos	KX529537	KX529628	KX529586	KX529498
Cochliomyia macellaria M077	Puerto Rico, Isla de Mona, Bajuras - Cerezos	KX529539	KX529627	KX529585	KX529495
Cochliomyia macellaria LA142	Saint Barts, Colombier Deciduos Dry Forest	KX529523	KX529631	KX529592	-
Cochliomyia macellaria LA096	Martinique, Cap de Macré Coastal Forest	KX529524	KX529626	KX529584	KX529491
Cochliomyia macellaria LA071	Dominica, Middleham Falls Trail	KX529525	KX529625	KX529583	KX529490
Cochliomyia aldrichi M080	Puerto Rico, Isla de Mona, Near Cueva Portugues	KX529529	KX529605	KX529563	KX529513
Cochliomyia aldrichi M085	Puerto Rico, Isla de Mona, Los Caobos	KX529530	KX529606	KX529564	KX529515
Cochliomyia aldrichi M086	Puerto Rico, Isla de Mona, Camino del Indio	KX529531	KX529607	KX529565	KX529514
Cochliomyia aldrichi M103	Puerto Rico, Isla de Mona, Los Caobos	KX529532	KX529608	KX529566	KX529516
Cochliomyia aldrichi M105	Puerto Rico, Isla de Mona, Near Cueva Portugues	KX529533	KX529609	KX529567	KX529518
Cochliomyia aldrichi M107	Puerto Rico, Isla de Mona, Near Cueva Portugues	KX529534	KX529610	KX529568	KX529517
Cochliomyia minima CU046	Cuba, Guantanamo, Alejandro de Humboldt National Park	KX529547	KX529633	KX529595	KX529510
Cochliomyia minima CU022	Cuba, Pinar del Rio, Viñales National Park	KX529549	KX529632	KX529593	KX529511
Cochliomyia minima CU023	Cuba, Pinar del Rio, Viñales National Park	KX529550	-	KX529594	KX529508
Cochliomyia minima DR136	Dominican Republic, Puerto Plata	KX529548	KX529635	KX529597	KX529509
Cochliomyia minima DR055	Dominican Republic, Haitises National Park	KX529552	KX529634	KX529596	KX529507
Cochliomyia minima PR141	Puerto Rico, Loiza, Mangrove area	KX529551	-	KX529600	KX529512
Cochliomyia minima PR132	Puerto Rico, Loiza, Mangrove area	KX529553	KX529636	KX529598	-
Cochliomyia minima PR133	Puerto Rico, Vieques, Monte Pirata	KX529554	KX529637	KX529599	KX529506
Cochliomyia hominivorax CO001	Colombia, El refugio Dry Forest	-	KX529611	KX529569	KX529482
Cochliomyia hominivorax CU020	Cuba, Pinar del Rio, Viñales Nacional Park	-	KX529612	KX529570	KX529483

Species name –Voucher Number	Location	*CO1*	*EF-1α*	*ITS2*	*28S rRNA*
Cochliomyia hominivorax CU033	Cuba, Pinar del Rio, Viñales Nacional Park	KX529556	KX529613	KX529571	KX529484
Cochliomyia hominivorax DR042	Dominican Republic, Rabo de Gato	KX529557	KX529614	KX529572	KX529485
Cochliomyia hominivorax DR105	Dominican Republic, East National Park, Yuma	KX529558	KX529615	KX529573	KX529486
Chrysomya megacephala FL003	USA, Florida, Everglades National Park, North-east	KX529521	KX529603	KX529561	KX529480
Chrysomya rufifacies CU004	Cuba, Granma: Turquino National Park	KX529555	KX529604	KX529562	KX529481
Hemilucilia sp. CO018	Colombia, Santander, Chipatá, Finca el Castillo	KX529560	KX529638	KX529601	KX529519
Lucilia cuprina PR073	Puerto Rico, Trujillo Alto, Ciudad Universitaria	KX529559	KX529639	KX529602	KX529520
Compsomyiops fulvicrura	As Published (Kutty et al. 2008)	FJ025607	FJ025667	-	FJ025504
Compsomyiops callipes	As Published (Wells and Sperling 2001)	AF295549	-	-	-

*The sample from Mexico was collected by Fabián García Espinoza from *Universidad Antonio Narro Unidad Laguna.*

PCR amplification and sequencing

We amplified regions of three nuclear loci: the protein coding elongation factor-1 alpha (EF-1α), the ribosomal 28S, and internal transcribed spacer 2 (ITS2), plus the mitochondrial protein coding cytochrome oxidase I (COI). The primer sequences are listed in Table 2. Protocols for COI reactions included an initial denaturation step of 95 °C for 2 min, followed by 35 cycles of 95 °C for 30 s, 44 °C for 45 s and 72 °C for 45 s, and a final elongation step of 72 °C for 10 min (Agnarsson et al. 2007). For ITS2 an initial denaturation step of 94 °C for 2 min was followed by 38 cycles of 94 °C for 30 s, 44 °C for 35 s and 72 °C for 30 s, and a final elongation step of 72 °C for 3 min (Agnarsson 2010). For EF-1α an initial denaturation of 95 °C for 5 min was followed by 35 cycles of 94 °C for 30 s, 55 °C for 35 s and 72 °C for 1 min, and a final elongation step of 72 °C for 10 min (McDonagh et al. 2009). For 28S rRNA initial denaturation of 94 °C for 5 min was

Table 2. PCR primers use in this study.

Gene	Primer name	Sequence (5' to 3')	Source
COI	LCO1490	GGTCAACAAATCATAAAGATATTGG	Folmer et al. (1994)
	CI-N-2776	GGATAATCAGAATATCGTCGAGG	Hedin and Maddison (2001)
EF-1α	B1	CCCATYTCCGGHTGGCACGG	McDonagh et al. (2009)
	C1	CTCTCATGTCACGDACRGCG	McDonagh et al. (2009)
28S	D1.F	CCCCCTGAATTTAAGCATAT	Friedrich and Tautz (1997)
	D35.486.R	TCGGAAGGAACCAGCTACTA	Friedrich and Tautz (1997)
ITS	ITS4	TCCTCCGCTTATTGATATGC	White et al. (1990)
	ITS5.8	GGGACGATGAAGAACGCAGC	Agnarsson (2010)

followed by 35 cycles of 93 °C for 1 min, 60 °C for 1 min and 72 °C for 2 min, and a final elongation step of 72 °C for 3 min (Friedrich and Tautz 1997). Amplified fragments were sequenced in both directions by University of Arizona Genetics Core. Sequences were interpreted from chromatograms using Phred (Green and Ewing 2002) and Phrap (Green 1999, Green and Ewing 2002) using the Chromaseq module (Maddison and Maddison 2010a) in the evolutionary analysis program Mesquite 3.03 (Maddison and Maddison 2010b) with default parameters. The sequences were then proofread by examining chromatograms by eye. Alignments were done using MAFFT (Katoh et al. 2002) through the online portal EMBL-EBI. The gene matrices were then concatenated in Mesquite 3.03 (Maddison and Maddison 2010b) and the full aligned data set is 3368 bp.

Phylogenetic analysis

We partitioned each gene and codon position for a total of eight partitions that were exported from Mesquite for model choice and the appropriate models were chosen using jModeltest v2.1.4 (Posada and Crandall 1998), and the AIC criterion (Posada and Buckley 2004). The corresponding model of evolution was used for the Bayesian analysis: GTR + Γ + I for 28S, ITS2 and COI3rd, GTR + Γ for COI1st, COI2nd, EF-1α3rd, HKY + Γ for EF-1α2nd and F81 for EF-1α1st. We ran the MC^3 (Metropolis Coupled Markov Chain Monte Carlo) chain in MrBayes v3.2.3 (Huelsenbeck and Ronquist 2001) through the online portal Cipres Science Gateway v3.3 (Miller et al. 2010). The analysis was run for 30.000.000 generations, sampling every 1000 generations. Chain stationary, ESS, and appropriate burnin was verified using Tracer 1.6 (Rambaut and Drummond 2009). Maximum likelihood (ML) analysis of the concatenated matrix was done in Garli (Zwickl 2006) using the same partitioning scheme and models.

Results

The phylogenetic analyses of the concatenated matrix, either using Bayesian or maximum likelihood approaches, recovered a generally well supported monophyletic *Cochliomyia* (Fig. 1). *Cochliomyia macellaria*, *C. hominivorax* and *C. minima* were recovered as monophyletic, while *C. aldrichi* was recovered as paraphyletic.

Independent analyses of 28S and ITS2 supported the monophyly of *Cochliomyia*, while COI and EF-1α recovered it as a paraphyletic group (Suppl. material 1). At the species level, EF-1α and 28S had limited signal and did not distinguish between *C. minima* and *C. aldrichi*. COI recovered the monophyly of *C. minima*, but did not resolve relationships among *C. aldrichi* and *C. macellaria*. ITS2 fully resolved the relationships within *Cochliomyia*, and is the only gene that recovered the monophyly of *C. aldrichi*. Despite of the incongruence detected among the four gene trees, they all recovered monophyletic *C. hominivorax* and three of the four genes (COI, 28S and ITS2) strongly supported a monophyletic *C. hominivorax* as sister to the other three species.

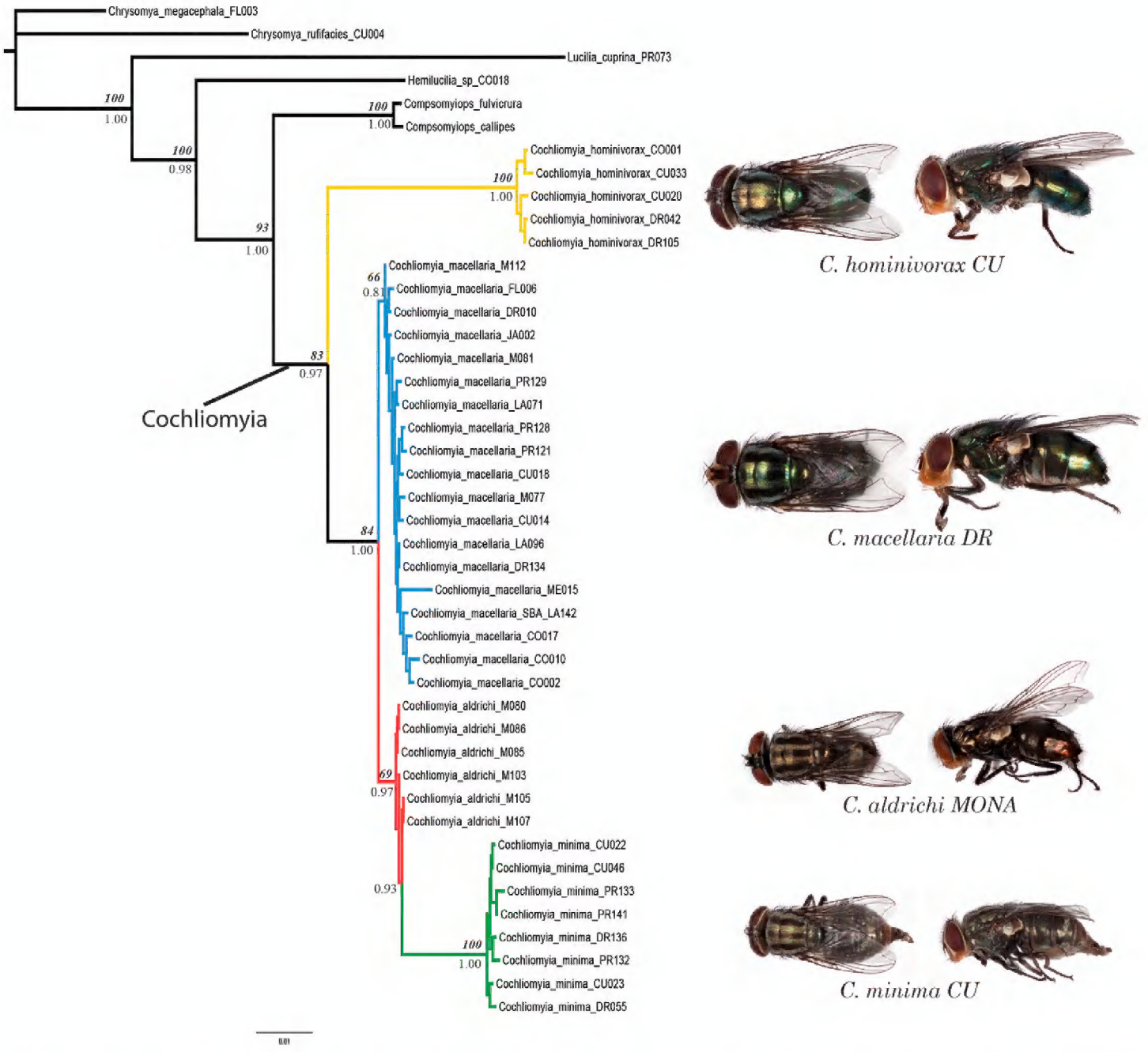


Figure 1. Phylogenetic relationship within *Cochliomyia* (ingroup) based on partitioned Bayesian analysis of the combined gene (COI, EF-1α, 28S rRNA and ITS2) data set. Branch support values: normal fond, Bayesian posterior probability; bold-italic font, maximum likelihood percentage bootstrap. Each color represents different species.

The concatenated dataset yielded a topology supporting a close relationship between *C. minima* and *C. aldrichi* which is congruent with the current taxonomy and indicates *C. macellaria* as the sister lineage of these two.

Discussion

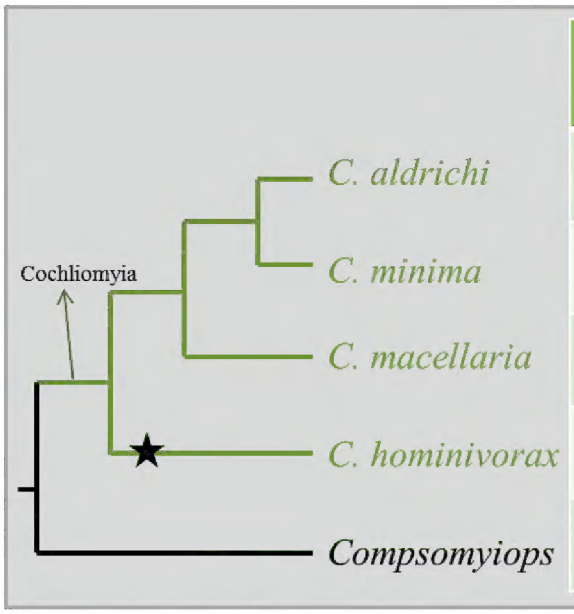
We present the first species complete phylogeny of the genus *Cochliomyia* including samples collected throughout the Caribbean from 13 different localities (Table 1). The concatenated matrix recovered a monophyletic *Cochliomyia*, partially resolved relationships among its species and recovered *Compsomyiops* as its sister group (Fig. 1), in congruence with prior studies (McDonagh and Stevens 2011, Singh and Wells 2011).

Independent gene trees did not yield fully congruent relationships among species, unsurprising as genes have independent histories. Two nuclear genes, 28S and ITS2 (adjacent loci), strongly supported the monophyly of *Cochliomyia* while the other two genes, COI and EF-1α did not. These results differ from McDonagh (2009), where EF-1α and COI strongly supported the monophyly of *Cochliomyia*, while 28S recovered *Cochliomyia* as paraphyletic. However, McDonagh (2009) included only two of the species of *Cochliomyia* represented by one specimen each. The differences between the studies could be due to a variety of taxon sampling issues, where our sampling was designed specifically to test monophyly and relationships among *Cochliomyia* species.

The monophyly of *C. hominivorax* is supported in all analyses, however, independent gene trees were not congruent with regards to other species. The relatively slowly evolving nuclear genes EF-1α and 28S supported *C. macellaria* but failed to distinguish between *C. minima* and *C. aldrichi*. The relatively rapidly evolving COI "DNA barcode" was found suitable for species identification and delineation (Hebert et al. 2003). COI was the only gene that recovered the monophyly of *C. minima*, however, COI did not resolve relationships among specimens of *C. aldrichi* and *C. macellaria*. This is surprising as these species are clearly identifiable based on morphological characteristics (Hall 1948, Whitworth 2010). Other studies also reported similar results where COI failed to distinguish among some closely related calliphorids (Wallman and Donnellan 2001, Nelson et al. 2007, Whitworth et al. 2007, Harvey et al. 2008, DeBry et al. 2013, Whitworth 2014), a result that has been attributed to incomplete lineage sorting. Results from COI, EF-1α, and 28S combined suggested *C. aldrichi* as sister to *C. macellaria*, instead of to *C. minima* as we would expect based on morphological characteristics. Based on these results we opted to add the rapidly evolving nuclear marker, ITS2 to help resolve species level relationships (Nelson et al. 2007, Agnarsson 2010). ITS2 was the only gene that recovered *C. aldrichi* as a monophyletic group and supported *C. minima* as its sister lineage.

Despite the incongruence detected between the four genes, a concatenate matrix recovered the monophyly of *C. hominivorax*, *C. macellaria* and *C. minima*, and supported the monophyly of *C. minima* plus *C. aldrichi*, mostly congruent with the current taxonomy. However, we found that *C. minima* is nested within *C. aldrichi*. That one species is paraphyletic with respect to another is not unexpected and does not necessarily refute their species status. The non-monophyly of *C. aldrichi* is surprising in that all specimens included in this study were collected from the tiny Mona Island (22 square miles). This indicates incomplete lineage sorting, or possibly recent speciation, rather than other processes like gene flow among species (given Mona's isolation, expectation of panmixia among *C. aldrichi* on the tiny island, and absence of *C. aldrichi* from other islands sampled). In contrast, *C. macellaria* and *C. minima* are present on most of the islands (Table 1) and the populations in different islands do not show any geographic structure (Fig. 1), indicating a constant gene flow among populations through migration.

The variability in feeding habits, habitat preference and morphology within *Cochliomyia* is considerable (Fig. 2). In feeding habits, *C. aldrichi*, *C. minima* and *C. macellaria* share similar behaviors. They are primarily carrion feeders, commonly found on



Feeding habits	Habitat preference	Oral spiracle pigmented as a spine	Adult Size (mm)	Distribution
● ■	Dry and hot	Unknown	5.5-7.0	West Indies (endemic)*
● ▲	Humid and cold**	present	5.5-8.5	West Indies (endemic)
● ■	Humid and warm	Absent	6-9	Nearctic & Neotropical
★	Humid and warm	Absent	8-10	Nearctic & Neotropical
● ■	Humid and warm	Present	6-12	Worldwide

Figure 2. Variability in feeding habits, habitat preference and morphology within *Cochliomyia*.
**C. aldrichi* has been reported in the Florida Keys Islands.
**We refer to temperatures around 10–15 °C.
● Carrion feeder; ▲ primary facultative parasite; ■ secondary facultative parasite; ★ obligate parasite.

decomposing cadavers. However, they are also capable of producing myiasis in open wounds as secondary facultative parasites under certain conditions or as primary facultative parasites as in the case of *C. minima*, (Hall 1948, Dear 1985). In contrast, *C. hominivorax* is an obligate parasite of mammals never found in decaying meats (Hall 1948, Stevens and Wallman 2006, McDonagh et al. 2009, McDonagh and Stevens 2011, but see Brody and Knipling 1943). Several authors have studied the evolution of parasitism within Calliphoridae and have concluded that the parasitic behavior in this family evolved independently several times (Stevens and Wallman 2006, McDonagh and Stevens 2011, Singh and Wells 2011). Within *Cochliomyia*, we conclude that parasitism evolved once in *C. hominivorax*, since the congeners are carrion feeders, as are members of the sister group, *Compsomyiops* (Fig. 2).

The habitat preferences of *C. hominivorax* and *C. macellaria* are largely known (Hall 1948, Greenberg 1971, Smith 1986, Wells and Greenberg 1992, Byrd and Butler 1996, Byrd and Castner 2010, Koller et al. 2011), however, little is known about *C. minima* and *C. aldrichi*. In recent studies of *C. minima* in Puerto Rico, Yusseff-Vanegas (2014) reported that *C. minima* prefer highly humid areas and can tolerate relatively cool conditions at altitudes >800m, while this species is absent from extremely dry and hot areas. Similar results were found in Dominican Republic and Cuba where *C. minima* was found abundantly in tropical and subtropical rain/moist forest even at altitudes >1300m, but absent from dry forest (unpublished data). These results supported the assumption that *C. minima* prefer humid cool areas, however, more studies are needed to understand its habitat preferences. In contrast, *C. aldrichi* seems to prefer hot dry areas, different from what we expected given the apparent recent divergence between *C. minima* and *C. aldrichi*. This is the case of recently divergent species like *Lucilia sericata* (Meigen) and *L. cuprina* Wiedemann, and *L. coeruleiviridis* Macquart and *L. mexicana* Macquart that have similar habitat preferences (Stevens and Wall 1996,

1997, Whitworth 2006, Byrd and Castner 2010, Whitworth 2010, 2014). However, *C. aldrichi* was found only on Mona Island, a subtropical dry forest with an average annual temperature of 27 °C (National Oceanic and Atmospheric Administration - NOAA) and low humidity through the year, strikingly different from *C. minima*. Yet, similar results have been reported before for closely related species like *C. megacephala* and *C. pacifica* (Singh et al. 2011) which are characterized by very different habitat preferences (Kurahashi 1981, 1991). Despite we have extensively collected in Florida (Everglades and the Keys), Cuba, Puerto Rico and the Bahamas, where *C. aldrichi* was previously reported (Whitworth 2010), we did not find this species. This could be explained by sampling bias as we only collected during the summer when precipitation and relative humidity are very high in the Caribbean. It is possible, for example, that *C. aldrichi* may be seasonal, being present during the winter when conditions are generally drier and cooler in the Caribbean. Alternatively, our sampling might indicate the recent extinction of this species from areas outside Mona, nevertheless, further studies are necessary to test these alternative hypotheses.

Two of the four species, *C. minima* and *C. aldrichi* are Caribbean endemics while the other two are widespread (Figs. 1–2). It is difficult to assess the biogeographical history of widespread species, however, we can conclude from our data that divergence between *C. minima* and *C. aldrichi* probably occurred in the Caribbean after the area was colonized. Island colonization is sometimes accompanied by a reduction in dispersal abilities and such processes may have led to reduced gene flow among islands, and promoted the formation of the Caribbean endemics. Further phylogeographic/phylogenomic studies including more taxa from the Caribbean and the continents are necessary to assess the colonization history of the genus and the possible secondary loss of dispersal ability in this group.

Conclusions

We provide the first complete phylogeny of *Cochliomyia*, supporting its monophyly and placement within the subfamily Chrysomyinae. Given incongruence among gene trees and low level of information at the species level for slowly evolving genes, the resolution of the outstanding questions in *Cochliomyia* phylogeny will require more data rich approaches, such as those offered by NGS methods. Nevertheless, we advance knowledge on the phylogeny, distribution, and life history of these species that should prove useful in future research and in realizing the potential of these species as forensic insects.

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Supplementary material 1

Phylogenetic relationship within *Cochliomyia* (ingroup) based on a Bayesian analysis of nucleotide data from (a) 28S, (b) COI, (c) EF-1α and (d) ITS2

Authors: Yusseff-Vanegas S, Agnarsson I

Data type: molecular data

Explanation note: Numbers indicate posterior probability support values.

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